Supporting information

Probing Intermolecular Interactions within the Amyloid β Trimer Using a Tethered Polymer Nanoarray

Sibaprasad Maity†, Apurba Pramanik†, Yuri L. Lyubchenko*

Department of Pharmaceutical Sciences, University of Nebraska Medical Center, 986025 Nebraska Medical Center, Omaha, NE 68198, United States

† These authors contributed equally to this project

*** To whom may be correspondence Prof. Yuri L. Lyubchenko; email: ylyubchenko@unmc.edu**

Table of contents

Materials and Methods

Supplementary Figures and Tables

1. Synthesis of FNA Aβ(14-23) trimer construct and characterization

The FNA construct was synthesized on Mermade-12 oligonucleotide synthesizer using phosphoradimite (PA) chemistry with the standard 200 nmol protocol. A standard cycle of DMT removal-coupling-capping-oxidation-capping was used with the coupling time extended to 10 minutes. The polymer was synthesized on 3' protected biotin serinol CPG (controlled porous glass). At first DMT was removed by 3% dichloroacetic acid in dichloromethane and then the spacer 18 phosphoramidite was coupled using 5-Ethylthio-1H-Tetrazole as a catalyst. Oxidation was performed with 0.02 M iodine in water/pyridine/THF (1:2:7) to produce a stable phosphate linkage. The capping was performed in acetic anhydride/2,6-lutidine/THF (1:1:8) and 16% v/v N-methyl imidazole in THF to protect the unreacted –OH groups from being involved in further reactions. After coupling of four spacer S18 units, the click chemistry reagent dibenzocyclooctyl (DBCO-dT-CE) phosphoramidite was added. Then following units were added; six units of spacer S18, one unit DBCO-dT-CE, six units spacer S18, one unit DBCO-dT-CE and four units of spacer S18. Finally, a thiol protected PA group at 5' was added at the end. The FNA construct was washed several times with acetonitrile before further immobilization of Aβ (14-23) peptides. Calculated amount of azide terminated Aβ (14-23) peptides were dissolved in 10 mM sodium phosphate buffer (pH 7.4) CPG was dispersed in it (molar ration FNA to peptide 1:6). The reaction was continued with stirring for 4 hours in dark. Un-reacted peptides were filtered off and CPG was washed three times with 10 mM sodium phosphate buffer, followed by with deionized water. Aβ (14-23) conjugated FNA was then cut off from CPG with 30% ammonium hydroxide for overnight. CPG was filtered off and solution containing the final product was collected. Ammonia was removed by vacuum for 4 hours. The final product was purified by reversed phase high performance liquid chromatography (RV-HPLC) (Solvent A: acetonitrile, solvent B: 0.1 M triethylammonium bicarbonate pH 7.5 buffer, Gradient 10–50% in 30 minutes, Phenomenex Gemini C18 column, 5μ, 250×4.6 mm). The product peak was collected and concentrated. This sample was run through analytical RV-HPLC to check the purity. Figure S1 shows only one peak, ensuring high purity of product. The product was further characterized by Matrix assisted Laser Desorption/ionization-time of flight (MALDI-Tof) mass spectroscopy using 3-Hydroxypicolinic acid as matrix. This highly pure FNA Aβ (14-23) trimer construct was used AFM force measurements.

2. Immobilization of streptavidin on mica surface and AFM imaging

Freshly cleaved mica surface (0.5 cm x 0.5 cm, approx.) was treated with 167 μM APS solution for 30 minutes, followed by rinses with deionize (DI) water. Surface was then covered with 5 μL of 0.05% aqueous glutaraldehyde for 1 hour, followed by rinses with DI water. Finally, surface was treated with 5 μL of 0.001μg/mL streptavidin solution in 10 mM sodium phosphate buffer (pH 7.4) for another 1 hour and then washed with DI water. Streptavidin coated surface was dried with argon gas and imaged in

Nanoscope III (Bruker Corporation, USA) in Tapping mode using AFM probes (TESPA, nominal spring constant 42 N/m; Bruker Corporation, USA). The images were processed with Femtoscan Online AFM software (Advanced Technologies Center, Lomonosov Moscow State University, Moscow). Figure S2A shows the scheme for functionalization procedure, Figure S2B showing AFM image of the surface. Volume of the proteins were measured using Enom features analysis and histogram was constructed as shown in Figure S2C, showing maximum peak at 93 nm^3, which is close to expected volume of streptavidin homo-tetramers (M.Wt. 52 kDa).

3. Control force measurement

(a) *Force measurements with FNA that contains no Aβ (14-23) peptide*: Small portion of synthetic FNA with three DBCO groups were cleaved from CPG, purified and confirmed by MALDI-Tof mass spectroscopy. This FNA does not contains any Aβ (14-23) molecules. For control experiment this FNA containing three DBCO (no peptide) was attached to AFM tip similar to main experiment. In brief, tip was washed with ethanol, water, dried and treated with UV. Tip was then dipped in 10 µM APS solution for 30 minutes and washed with water. The tip was treated with 100 µM GMBS solution in DMSO for 1 hour, followed by washing with DMSO and DI water. After that, the tip was treated with 10 nM of FNA containing TCEP solution for overnight and followed by washing with DI water. DBCO groups on tip were then treated with 100 µM of Azide-PEG_4-MAL for 1 hour, followed by washing with DI water. The maleimide groups were then converted to OH by dipping the tip in 10 mM β-mercaptoethanol solution for 30 minutes, followed by washing with DI water. Mica surface was functionalized with streptavidin as discussed above. Force-distance experiments were performed with those (Figure S3A). Thousands of FD curves were collected to achieve enough data that showing successful binding events. In this data set we did not found any extra peak, except biotin-streptavidin unbinding events. Overlap plot of several FD curves are shown in Figure S3B indicating nice reproducibility. The force peaks were fitted with WLC model to estimate force and contour length. Figure S3C shows force distribution for biotin-streptavidin complexes having maximum at 86 ± 2.1 pN. Contour length histogram (Figure S3D) shows peak maxima at 41 ± 0.3 nm. This value is close to our designed FNA tether contour length (40 nm).

(b) *Testing unspecific binding of FNA Aβ (14-23) trimer functionalized AFM tip with substrate:* The AFM tip was functionalized with FNA Aβ (14-23) trimer as to procedure discussed in main text (Materials and Method section) and mica surface was functionalized with amino groups using 167 µM APS solution. Force spectroscopy was performed with those tip and surface. Our results showed only 0.1% of rupture force events with the force in the range of ~15-20 pN in distances as short as ~10-15 nm. During data analysis of our main experiments we did not consider these unspecific events.

4. Reproducibility of experimental data

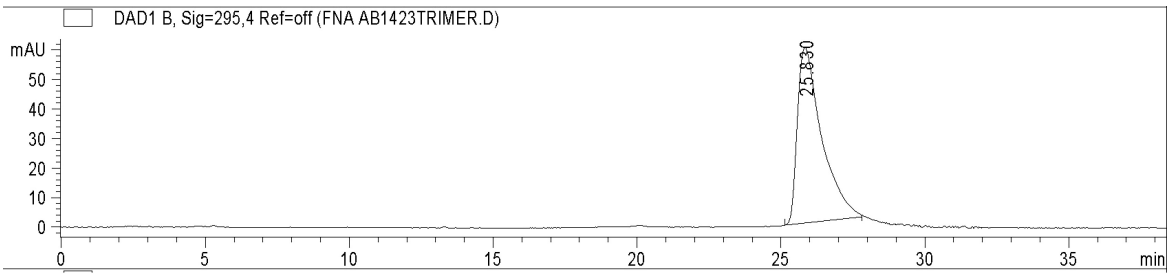
To ensure that our measurements are correct, we repeated all the experiments two times at similar experimental conditions. The results demonstrating the reproducibility are shown in Tables S1 and S2.

Table S1. Reproducibility of force measurements; analysis of Aβ (14-23) trimer; values are obtained by fitting corresponding histograms with Gaussian functions. Values are shown as mean ± S.E.M.

Expt. no.	Peak1		Peak2		Peak3	
	F (pN)	Lc (nm)	F (pN)	Lc (nm)	F (pN)	Lc (nm)
1	48 ± 2.4	18 ± 0.6	53 ± 3.2	30 ± 0.4	87 ± 7.4	42 ± 0.6
2	44 ± 1.2	17 ± 0.2	50 ± 1.0	29 ± 0.3	84 ± 3.6	42 ± 0.4

Table S2. Reproducibility of force measurements; analysis of Aβ (14-23) dimers; values are obtained by fitting corresponding histograms with Gaussian functions. Values are shown as mean ± S.E.M.

	Dimer (two peaks close to each other)				Dimer (two peaks far from each others)			
Expt. no.	Peak 1		Peak 2		Peak 1		Peak 2	
	F(pN)	Lc(nm)	F(pN)	Lc(nm)	F(pN)	Lc(nm)	F(pN)	Lc(nm)
1	44 ± 2.0	28 ± 0.5	74 ± 5.7	42 ± 0.8	37 ± 2.8	18 ± 0.6	84 ± 4.8	44 ± 0.6
2	43 ± 1.6	27 ± 0.4	78 ± 3.6	42 ± 0.4	48 ± 1.9	17 ± 0.7	89 ± 4.7	40 ± 0.5



Signal 2: DAD1 B, Sig=295,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	25.830	BB	0.8202	3496.04199	59.44093	100.0000
Totals :				3496.04199	59.44093	

Figure S1. Analytical HPLC data shows high purity of FNA Aβ (14-23) trimer construct, which was confirmed by mass spectroscopy. This highly pure sample was used for force spectroscopy experiments.

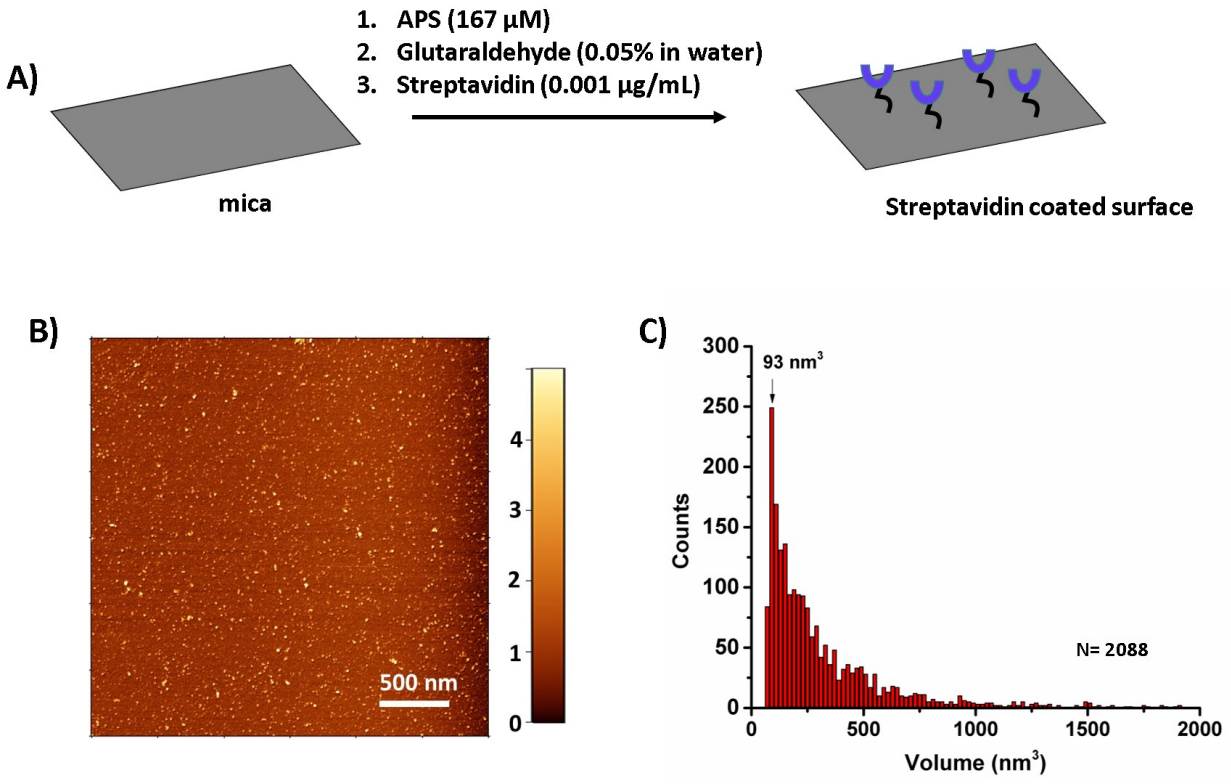


Figure S2. Characterization of streptavidin coated surface; (A) Scheme showing protocols for immobilization of streptavidin proteins with the mica surface. (B) AFM image showing distribution of proteins onto the surface (C) Volume distribution for streptavidin.

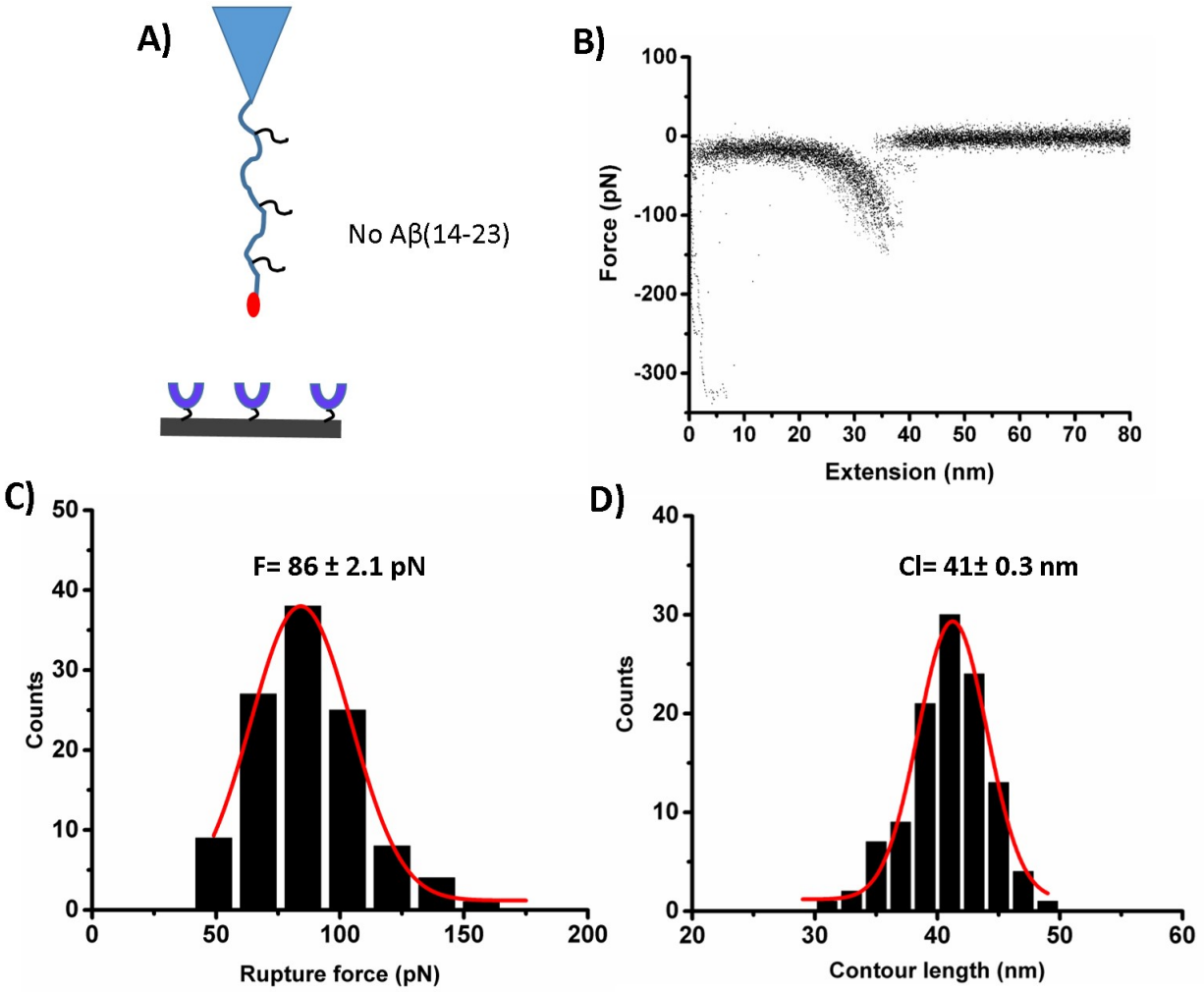


Figure S3. Control force experiment, biotin containing FNA (no attached peptides) were probed with streptavidin. (A) Schematic of experimental set up. (B) Overlap plot of several force-extension traces showing reproducibility of data. (C) and (D) Force and contour length histogram for biotin-streptavidin interactions. Histograms were fitted with Gaussian function (red). Values are shown as mean ± S.E.M. Number of data (N)= 112.

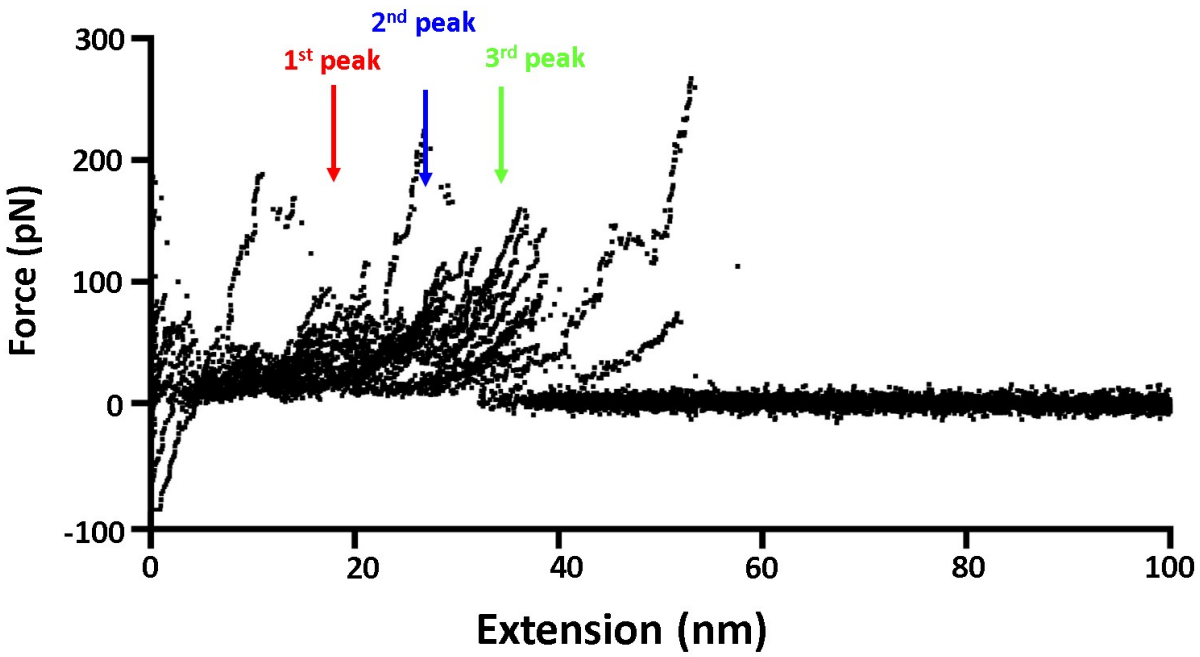


Figure S4. Overlay of force-distance curves containing three peaks. Number of curves used 21.

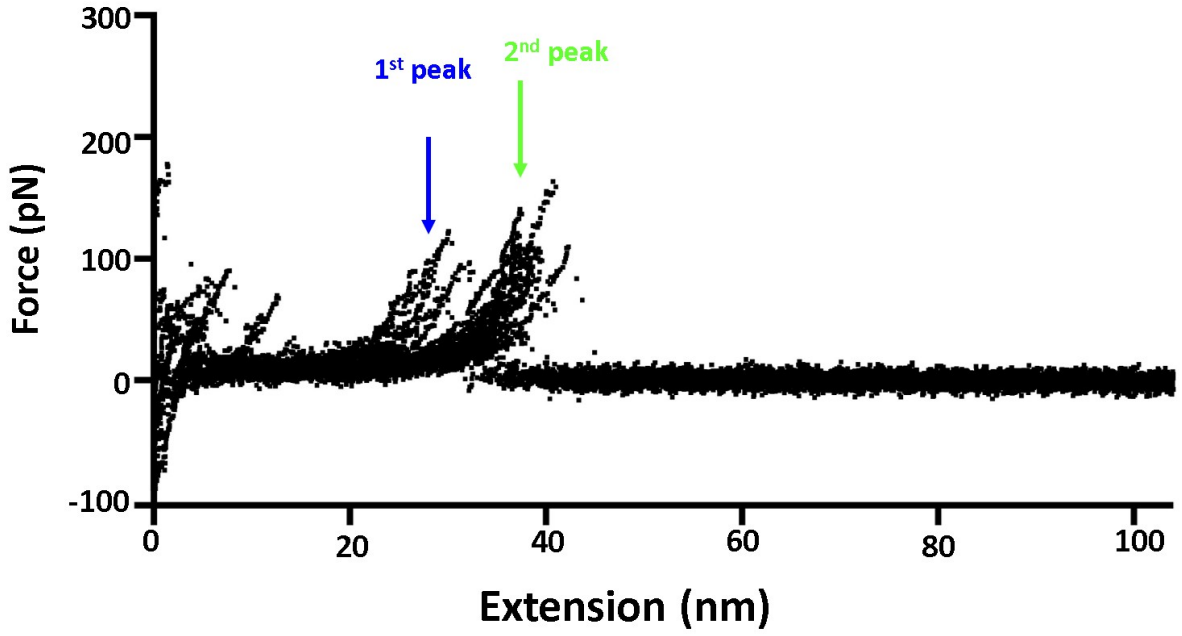


Figure S5. Overlay of force-distance curves containing two peaks close to each other. Number of curves used 20.

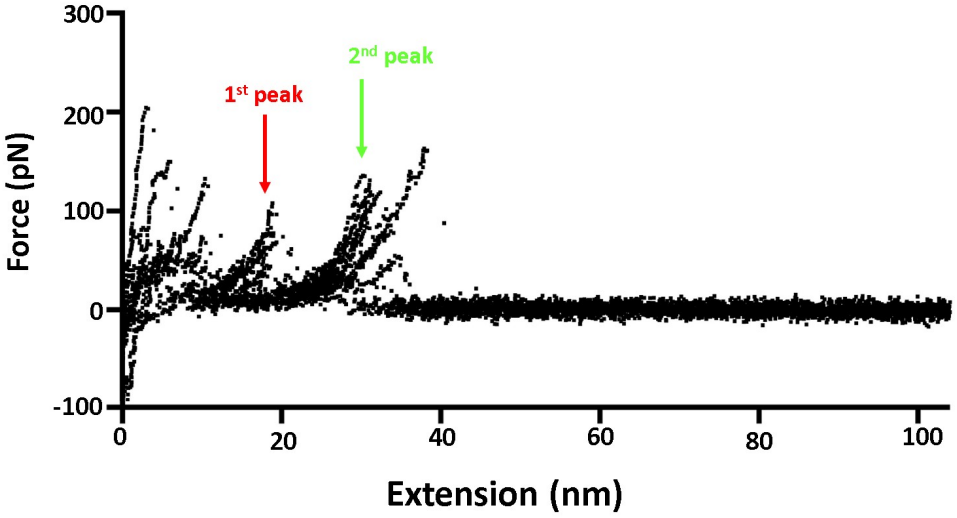


Figure S6. Overlay of FD curves containing two peaks separated from each other a long distance. Number of curves used 20.

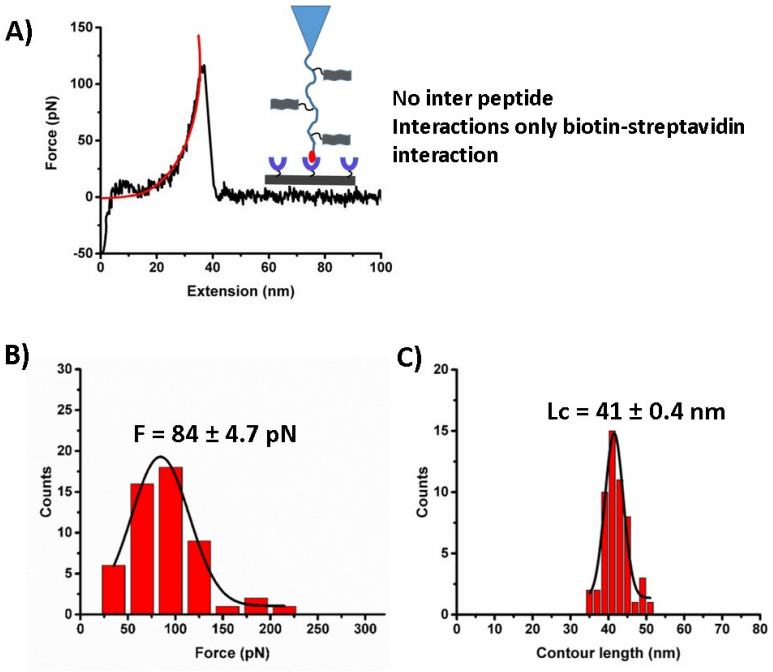


Figure S7. Peptides had no interaction with each other, only biotin-streptavidin complex forms. (A) A typical FD curve fitted with WLC model (red). The inset shows experimental set up. (B) Force histogram fitted with Gaussian function (black). (C) Contour length histogram fitted with Gaussian function (black). Values are shown as mean ± S.E.M. Number of data point = 54.